LAWRENCE
LIVERMORE
NATIONAL
LABORATORY

LLNL-JRNL-403909

Magnetic Bead Based Immunoassay for Autonomous Detection of Toxins

Y. Kwon, C. A. Hara, M. G. Knize, M. H. Hwang, K. S. Venkatesteswaran, E. K. Wheeler, P. M. Bell, R.. F. Renzi, J. A. Fruetel, C. G. Bailey

May 15, 2008

Analytical Chemistry

Disclaimer

Magnetic Bead Based Immunoassay for Autonomous Detection of Toxins

Youngeun Kwon+[§], Christine A. Hara+[§], Mark G. Knize[§], Mona H. Hwang[§], Kodumudi S.Venkatesteswaran[§], Elizabeth K. Wheeler[§], Perry M. Bell[§], Ronald F. Renzi[‡], Julie A. Fruetel [‡], Christopher G. Bailey*[§]

[§] Lawrence Livermore National Laboratory, Livermore, California

[‡] Sandia National Laboratories, Livermore, California

+These two authors contributed equally.

*Corresponding author. E-mail: bailey38@llnl.gov. Phone: 925-422-0578. Fax: 925 422-4100.

ABSTRACT

As a step towards toward the development of a rapid, reliable analyzer for bioagents in the environment, we are developing an automated system for the simultaneous detection of a group of select agents and toxins. To detect toxins, we modified and automated an antibody-based approach previously developed for manual medical diagnostics that uses fluorescent eTag™ reporter molecules and is suitable for highly multiplexed assays. Detection is based on two antibodies binding simultaneously to a single antigen, one of which is labeled with biotin while the other is conjugated to a fluorescent eTag™ through a cleavable linkage. Aqueous samples are incubated with the mixture of antibodies along with streptavidin-coated magnetic beads coupled to a photo-activatable porphyrin complex. In the presence of antigen, a molecular complex is formed where the cleavable linkage is held in proximity to the photo-activable group. Upon excitation at 680 nm, free radicals are generated, which diffuse and cleave the linkage, releasing the eTags™. Released eTags™ are analyzed using capillary gel electrophoresis with laser-induced fluorescence detection. Limits of detection for ovalbumin and botulinum toxoid

individually were 4 ng/mL (or 80 pg) and 16 ng/mL (or 320 pg), respectively, using the manual assay. In addition, we demonstrated the use of pairs of antibodies from different sources in a single assay to decrease the rate of false positives. Automation of the assay was demonstrated on a flow-through format with higher LODs of 125 ng/mL (or 2.5 ng) each of a mixture of ovalbumin and botulinum toxoid. This versatile assay can be easily modified with the appropriate antibodies to detect a wide range of toxins and other proteins.

KEYWORDS: biological terrorism, botulinum toxin, eTag™, autonomous instrument,

There are many applications where continuous and automated analysis of pathogens and toxins in the environment is desirable[1-3]. In the case of bioterrorism, where it is not known which particular agent a terrorist might use to infect people and contaminate the environment, detection systems must be able to detect a large number of possible threat agents. Additional requirements for such systems include rapid reporting capability and very sensitive and highly selective detection. To address these needs, we are developing a system capable of detecting a wide range of aerosolized threats released into the environment. The system, called the BioBriefcase, is composed of a single aerosol collector that feeds three analysis trains performing independent, complementary assays to detect up to 100 bio-agents simultaneously. Each assay train targets specific groups of biological agents: PCR assays are used for the detection of bacterial pathogens and DNA viruses, reverse transcriptase PCR for RNA viruses, and immunoassays for toxins. To streamline and simplify the overall system design and operation, all three assay trains use a common fluorescent reporter-based assay, enabling each train to be coupled to a common detection platform, a custom-built miniaturized capillary gel electrophoresis (CGE) system with laser-induced fluorescence (LIF) detection.[4]

Antibody methods are well established for the sensitive detection of toxins such as Clostridium botulinum toxin. These methods include the use of capture and detection antibodies patterned on surfaces [5], magnetic bead capture [6], or single use plastic chips. [7] Other reported approaches include

microfluidic chip-based FRET technology [8], and an antibody functionalized AFM cantilever [9]. In order to be compatible with the overall system design and requirements for a robust, autonomous, and multiplex-capable instrument, we sought to develop a flow-through approach that could be automated and has the ability to run unattended for days or weeks at a time.

In this paper, we describe the development of a sensitive, selective, rapid, and multiplex immunoassay and its implementation onto an automated flow-through platform as one of the independent assay trains for the integrated BioBriefcase system. This assay is based on a protocol developed primarily for clinical applications using eTag™ reporter molecules.[10] eTag™ reporters are fluorescent small molecules, each of which has a uniquely defined electrophoretic mobility, rendering them separable from one another during CGE analysis. Antibodies specific to target proteins are labeled with unique eTags™, which are released by photolysis after the antigen-antibody complex is formed and are detected using laser induced fluorescence in the CGE analysis.

Detection of analytes using eTag™ reporter molecules offers a number of advantages. First, the recognition molecules used in the eTag™ assay can be easily modified to develop a versatile detection platform for a wide range of analytes. Moreover, the molecular structure of the eTags™ themselves can be modified to alter their electrophoretic mobility. This combination of features allows multiple eTags™, each associated with different antibodies, to be used simultaneously in a single assay. The eTags™ are separated from one another with CGE, enabling rapid detection of multiple analytes. Because the structure and electrophoretic mobility of each tag is well characterized, a fluorescence peak at a given elution time in the CGE analysis is a reliable indicator of the presence of the antigen in the sample. The accuracy of the analysis system is enhanced by running electrophoretic markers concurrently with the sample.

The benchtop protocol for the eTag™ immunoassay uses filtration and resuspension of components, which was not compatible with our desire for an autonomous system that could perform unattended, reproducible assays. To enable automation and provide increased detection sensitivity we

introduced streptavidin-coated magnetic beads into the assay. The magnetic bead-based assay enables an automated flow-through approach, allowing removal of un-reacted reagents and washing of the trapped products, as well as the ability to decontaminate the system between runs. We optimized this new protocol for detection of Clostridium botulinum toxoid A (BotTox) and ovalbumin (Ov) in manual assay format and transferred it to an automated platform.

Experimental Section

Materials. The proteins Ov and BotTox, and rabbit polyclonal antibodies that recognize either Ov or BotTox were purchased from the Critical Reagents Program (Frederick, MD). The sheep polyclonal antibody recognizing Ov was purchased from Cortex Biochem (San Leandro, CA). Goat polyclonal antibody for the recognition of BotTox was purchased from Tetracore Inc. (Rockville, MD). Purchased antibodies were conjugated to biotin or to an eTag™ by Monogram Biosciences (South San Francisco, CA). Each antibody has a specific eTag™, identified according to its "Pro" number, attached as shown in Table 1. Antibody solutions were diluted with 1x Assay Buffer (Monogram Biosciences) to 400 nM working stocks. Wash Buffer (1x), Assay Buffer (10x), Capillary Electrophoresis Standard (CES) (1000x) containing two electrophoretic markers (M1 and M2) and Scissors Reagent (5 mg/mL) were purchased from Monogram Biosciences. Dynabeads M-280, Streptavidin (10 mg/mL) and Tris·HCl (1 M, pH 8.0) were purchased from Invitrogen Corp. (Carlsbad, CA). An interferent panel (Table 2) containing 16 suspicious powders and 18 reference materials was purchased from the Critical Reagents Program (Frederick, MD).

Preparation of antibody solutions ("S2"): Antibody solutions for each assay were prepared in the following concentrations. Ov singleplex: 40 nM biotin-rabbit IgG anti-Ov, 40 nM Pro1-rabbit IgG anti-Ov. BotTox singleplex: 40 nM biotin-rabbit IgG anti-BotTox, 40 nM Pro11-rabbit IgG anti-BotTox.

Ov and Botox Duplex: 20 biotin-rabbit IgG anti-Ov, 40 nM Pro1-rabbit IgG anti-Ov, 20 nM biotin-rabbit IgG anti-BotTox, 40 nM Pro11-rabbit IgG anti-BotTox. Multiplex antibody solution: 40 nM Pro1-rabbit IgG anti-Ov, 20 nM biotin-rabbit IgG anti-Ov, 40 nM Pro12-sheep IgG anti-Ov, 20 nM

biotin-sheep IgG anti-Ov, 40 nM Pro11-rabbit IgG anti- BotTox, 40 nM biotin-rabbit IgG anti-BotTox, 40 nM Pro13-goat IgG anti-BotTox, and 40 nM Goat IgG anti-BotTox biotin in Assay Buffer.

Preparation of magnetic bead/scissors solution ("S3"): Dynabeads (75 μl for manual assays, 150 μl for automated assays) were washed in a 1.5 mL microcentrifuge tube using a magnetic particle concentrator. The magnetic pellet was allowed to form by placing microcentrifuge tubes containing magnetic beads on a magnetic particle concentrator and removing the soluble fraction containing unbound streptavidin with a pipette. The wash step was repeated three times using Assay Buffer (300 μl for manual, 600 μl for automated assays). The final bead pellet was resuspended in 960 μl of Assay Buffer and 40 μl of 5 mg/mL Scissors Reagent.

Manual assay for detection of ovalbumin: Manual assays were conducted in 96-well plates at room temperature. The 96-well plate was wrapped with aluminum foil during the assays to avoid loss of the light-sensitive scissors compound. For a typical assay, a 20 μl aliquot of sample (S1, 125 ng/mL or 2.5 ng) was mixed with 20 μl of antibody solution (S2, Ov singleplex antibody solution). The sample and antibody mixture was incubated with shaking. After the incubation, 20 μl of the scissors/streptavidin-coated magnetic bead mixture (S3) was added and incubated with shaking. The 96-well plate was then transferred to the magnetic particle concentrator causing the magnetic particles to adhere to the sides of the individual sample wells. The soluble fraction containing unbound antibodies was removed from the wells by pipetting. The pellets were washed three times with 100 μl wash buffer followed by resuspension in 25 μl of Tris buffer (2 mM Tris·HCl, pH 8) containing electrophoretic markers M1 and M2. The 96-well plate was removed from the particle concentrator and transferred to a 96-well light emitting diode plate illuminator with 680 nm excitation (Monogram Biosciences, South San Francisco, CA). The samples were illuminated for 5 minutes followed by shaking for an additional 5 minutes. While using the magnetic particle concentrator, the soluble fraction containing released eTags™ was transferred to a new 96-well plate, and was analyzed by capillary electrophoresis using the ABI3100 Genetic Analyzer (Applied Biosystems Foster City, CA).

Manual assay for detection of BotTox: The BotTox sample (125 ng/mL or 2.5 ng) was analyzed using the singleplex protocol for Ov but with the BotTox singleplex antibody solution (S2) solution.

Multiplex assay for detection of Ov and BotTox: Ov (125 ng/mL or 2.5 ng) and BotTox (125 ng/mL or 2.5 ng) were detected simultaneously using the standard manual protocol in conjunction with the multiplex antibody solution. Each antigen was also used individually with the multiplex antibody solution to check for false positives.

Interferent panel: The suspicious powders were diluted with water to a concentration of 10 mg/mL. Soluble interferents were used as received (1 mg/mL). Interferent suspension (1 μl) was added to an antigen sample containing 19 μl of 125 ng/mL Ov (or 2.38 ng). At these concentrations and volumes, the mass ratio of suspicious powders to Ov was approximately 4000:1 and approximately 400:1 for soluble interferents. The effect of each interferent on assay performance was tested using the singleplex assay protocol for Ov as previously described.

Automated fluidics instrument: A photograph of the custom fluidics instrument used in these experiments is shown in Fig. 1A. The instrument consists of a 10-port valve and reversible pump (both from Valco Instruments, modified by Global FIA, Fox Island, WA). A magnetic trap, diode illuminator, and vortex and shaking mixers were manufactured in house (Fig. 1A). These components are connected by 1/16 O.D. Teflon® tubing with internal diameters of 0.01, 0.02, or 0.03 inches. Pump carrier fluid was water with 1% Tween20 detergent (Sigma-Aldrich, St.Louis, MO). Reagents were a wash buffer (WB), assay buffer (AB), test sample (S1), antibody mixture (S2), magnetic beads and scissors compound mixture (S3), and Tris buffer (TB). These were positioned on the system as shown in the schematic drawing in Fig. 1B. The electronic components were integrated with a homemade circuit board and linked to a laptop computer running Labview Software (National Instruments Corp. Austin TX). The assays were protected from light by placing a light-insulating box over the fluidics instrument.

Instrument operation

To initiate the analysis, an air bubble (10 μl), sample S1 (20 μl), antibody solution S2 (20 μl) and a trailing air bubble (10 μl) are drawn into the transfer line. The reagents are mixed inside the tubing by a shaking motion provided by the tube mixer for 30 minutes. The reagent plug containing a mixture of S1 and S2 is moved back to the 10-port valve and, after 1 minute of vortex mixing, S3 (20 μl) is added to the leading edge of the mixture. This plug is moved back to the tube mixer and allowed to react while being shaken for 30 minutes. The antibody/antigen complex bound to magnetic beads is then captured by passing the reagent plug through a magnetic trap. The magnetic trap consists of a permanent magnet that can be swung into place adjacent to the flow tubing to pull the magnetic bead complexes from the solution to form a visible pellet attached to one side of the tubing. The pellet of magnetic beads is then washed by flowing wash buffer (150 μl) through the magnetic trap. Unbound antibodies and antigens are flushed from the system by this washing step. Tris buffer (2 mM, pH ~8) is flowed over the pellet to replace the wash buffer. The magnetic pellet is illuminated for 5 minutes using a single light-emitting diode. The solution of freed eTags™ is dispensed into a 0.2 mL microcentrifuge tube and transferred to a 96-well plate for analysis on the ABI 3100 Genetic Analyzer. The magnet is moved away from the side of the tubing to release the trapped beads during decontamination steps.

Results and Discussion

The assays described here are based on antibody binding, magnetic bead capture, photo-activated release, and detection of fluorescent eTags™ as shown in Scheme 1. We modified the standard protocol using eTags™ for use in our autonomous system by incorporating streptavidin-coated magnetic beads to enable magnetic trapping of the reagent complex, facilitating separation and washing and thus enabling automation of the assay. An aqueous test sample containing an antigen is mixed with a solution containing pairs of labeled antibodies capable of binding antigen molecules. One antibody in each pair is conjugated to an eTag™ through a cleavable linkage and the other is labeled with biotin to enable binding of the antibody-antigen complex to the streptavidin-coated magnetic beads. The complex captured by magnetic beads is isolated from the unreacted reagents and washed. The Scissors Reagent

is a porphyrin compound that generates free radicals upon illumination at 680 nm. The free radicals diffuse to cleave the linkage between the eTag™ and the antibody to release eTags™. The solution containing freed eTags™ is then analyzed without further processing using capillary gel electrophoresis with laser-induced fluorescence (LIF) detection.

The performance of the designed assay was tested using a toxin surrogate, ovalbumin (Ov), as a target. Figure 2 shows the resulting electropherograms from aqueous samples either with Ov (2B) or without Ov (2A). The Pro1 eTag™ peak, indicative of the presence of Ov antigen, is observed only in the sample containing Ov. Markers M1and M2 provide references for peak identification based on the relative migration time and are seen both in 2A and B. The markers also define the elution window in which all eTags™ should be seen. Peaks outside of the elution window, such as the one at 10 minutes in Figure 2B, are disregarded since their electophoretic mobility falls out side the range of the eTags™ used in this assay.

Determination of optimal incubation times

Optimal times for both incubation periods were investigated using samples containing Ov (125 ng/mL or 2.5 ng). The singleplex format for Ov was followed as previously described but with varying incubation times. The first incubation time (T1) of sample antigen (S1) and antibody solution (S2) tested at 5 minutes, 10 minutes, 20 minutes, 30 minutes, 40 minutes and 60 minutes while keeping the second incubation time (T2, antigen/antibody and magnetic bead mixture) constant (30 minutes). The experiment was repeated by varying T2 from 5 minutes to 60 minutes while keeping T1 constant (30 minutes). Results showed the greatest signal response at 30 minutes for T1 and for T2. However, no significant decrease in signal was detected from decreasing T1 to 10 minutes while keeping T2 at 30 minutes and these times were used for subsequent manual assays.

Limit of detection

The limit of detection (LOD) was determined using the singleplex benchtop assay format. Serial dilutions were made for each antigen by diluting stock solution with Assay Buffer. For Ov, the

response curve is shown in Figure 3 as the average of duplicate analyses. The decreasing signals seen above 800 ng/mL (or 16 ng) are due to the 'hook effect' commonly seen in immunoassay analyses at high analyte concentration.[11] In our case the effect is observed because of the reaction stoichiometry and the limiting amount of both the eTag™ labeled and biotin labeled antibodies, since each toxin molecule must capture one molecule of each antibody type to form the complex that must be magnetically captured and contain the eTag™. Even at 500 μg/mL (or 10 μg), however, a signal nearly three times background is seen, a response that could trigger a repeat analysis of that sample, or be considered a positive detection when using appropriate thresholds.

The LOD is taken as the analyte concentration at which the average fluorescent intensity is larger than three times that of the background. For Ov, the LOD is 4 ng/mL (or 80 pg). The LOD for BotTox in a similar experiment with appropriate antibodies, is 16 ng/mL (or 320 pg) (data not shown).

Comparisons of these results with those reported in the literature indicate our technology is competitive with current assays. For botulinum toxoid A and B, LODs of 40 and 200 ng/mL, respectively, have been reported [12]. For ELISA assays on a chip, the LOD was 2 ng/mL for toxoid A[7]. For Ov our singleplex assay demonstrates up to 2 orders of magnitude increased sensitivity over a reported fiber optic system[13].

Assay robustness

It is critical that a field instrument be insensitive to a wide range of environmental backgrounds and we therefore investigated the effect of the interferents listed in Table 2 on assay performance. Of the 34 interferents, none showed a detectable effect on the performance of the assay when added as previously described in the methods section. The effects of interferents have been reported with other antibody-based assays for toxin surrogates. Ligler et al. used interferents at 10 μg/mL tested against 100 ng/mL toxin, and reported no false positive responses and a reduction in signal intensity of approximately 50%[12]. Han et al. also examined the use of interferents and reported little effect on their

antibody-based assay[7]. Our results indicate our assay protocol is robust and is suitable for further development for a field deployable instrument.

Simultaneous detection of multiple analytes

In an environmental monitoring system where warnings would be issued in the event of a positive detection, it is critical that false positives be kept to a minimum. To achieve this, we use multiple independent antibodies coupled to different eTags™ to monitor for a single antigen. For a positive call to be made on the system all sets of antibodies must indicate the presence of the antigen. Using four different sets of antibodies previously described, we conducted simultaneous detection of two analytes to test the capability of the assay to detect multiple targets in the same sample. Reference peaks M1 and M2 were not used in these experiments. Figure 4A shows the electropherogram obtained from an Ov sample detected with a pair of eTags™, Pro 1 and Pro 12 coupled to antibodies as shown in Table 1. Figure 4B shows the result from a BotTox sample, detected with antibodies linked to eTags Pro 11 and Pro 13. The electropherogram in Figure 4C shows positive results from a sample containing both Ov and BotTox and all four antibodies. In each case, the electropherograms show that the peaks correspond to the appropriate antibodies; the peaks are observed only when the corresponding antigen is present in the sample, and the fluorescent peaks are negligible when antigen is absent. No significant nonspecific binding is observed in the samples, indicating high assay specificity.

Autonomous assay using computer controlled fluidics

Using conditions optimized for the manual duplex assays, we transferred the parameters to the automated instrument shown in Fig 1B. In the instrument, the antibody complex bound to streptavidin-coated magnetic beads is separated from the solutions by placing the flow path tubing adjacent to a permanent magnet. Illumination of the trapped magnetic beads released appropriate eTags™ and the eluted solution was analyzed offline with the ABI 3100 electrophoresis instrument. Figure 5 shows example electropherograms from samples processed using the automated instrument. 5A is the result from a blank sample, containing single antibodies, one recognizing Ov and one recognizing BotTox.

The peak for electrophoretic marker M1 is easily seen, however, M2 is smaller than expected. This is most likely caused by photobleaching of the reagent due to exposure to stray light in storage. In sample 5B, containing Ov and BotTox, large signals from eTags™ Pro 1 and Pro 11 are seen. Repeated sample processing (3x) showed relative standard deviations in the peak area of 18% and 8% for the Ov and BotTox peaks, respectively.

Total analysis time for the automated assay, including CGE was less than 100 minutes, which is consistent with our need for periodic sampling of environment. The LOD for the autonomous system was 125 ng/mL, or a total amount of 2.5 ng for both BotTox and Ov. In an unattended system it is critical that reagent consumption and waste generation be minimized. In our automated assay, the volume of waste generated was 3.5 mL to process the 20 μl liquid sample.

Conclusion

We have developed and tested a magnetic bead based immunoassay and implemented it onto a flow-through automated analysis system. The results show our assay is a useful diagnostic tool conferring several advantages over other assays for toxins. We showed the simultaneous sensitive and specific detection of multiple analytes from a single sample, with a relatively short analysis time. The dynamic range of all our assays span 2-4 orders of magnitude, and each assay can be used to declare a sample as "positive" (i.e., containing antigen molecules in a concentration above the limit of detection) over 4-5 orders of magnitude, from 16 ng to 500 µg per mL. No significant nonspecific binding is observed for any antigen in our assays, indicating a high degree of specificity. The multiplexed immunoassays can be used to rapidly screen for and identify potentially interfering or competing substances and can be used to both qualify and quantify cross-reactive binding. Automation using a pump, 10-port valve, magnetic trap and LED for sample processing showed successful assays in less than 100 minutes with an LOD of 125 ng/mL.

Work is in progress to increase the number of analytes that can be detected by the system and to integrate this assay with spore detection systems to produce a miniaturized unit for fully autonomous environmental monitoring .

Acknowledgements

This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344, with funding from the Department of Homeland Security. Sandia is a multiprogram laboratory operated by Sandia Corporation, a Lockheed Martin Company, for the United States Department of Energy under contract DE-AC04-94AL85000.

FIGURE CAPTIONS

Figure 1. Photograph of the immunoassay fluidics unit containing a pump, 10-port valve, capture magnet and illumination devices (A). Schematic illustration of the fluidics components representing their arrangement and relative locations (B).

Figure 2. Electropherograms showing the detection of ovalbumin using a manual assay format.

A blank sample (A) and a sample containing Ov (B) are analyzed using antibody solutions containing rabbit IgG antibodies that recognize Ov. The fluorescent signal from the corresponding reporter molecule (Pro1) is observed from only the electropherogram of a positive assay.

Figure 3. Chart of detection range for Ovalbumin (Ov).
The assay is performed using samples containing Ovalbumin in a broad range of concentrations using the manual magnetic bead based assay and shows a limit of detection is 0.004 μg/mL.

Figure 4. Electrophereograms showing multiplexed detection of toxins using the magnetic bead based immunoassay. Figure 4A shows the detection of Ov with sheep and rabbit antibodies, 4B shows the detection of BotTox with rabbit and goat antibodies. Figure 4C shows the multiplexed detection of both Ov and BotTox, and all four eTag™ peaks.

Figure 5. Autonomous detection of Ov and BotTox from single antibody detection. 5A shows a blank sample, 5B shows 125 ng/ml of Ov and BotTox with single eTag™ antibodies.

SCHEME TITLES

Scheme 1. Reaction scheme of the magnetic bead-based immunoassay.

Table 1. Components of multiplexed immunoassays

Analyte	Capture Antibody	Detection Antibody
Ovalbumin (Ov)	Biotin-Rabbit IgG anti-Ov	Pro1-rabbit IgG anti-Ov
		Pro12-sheep IgG anti-Ov
Botulinum Toxoid A (BotTox)	Biotin-Rabbit IgG anti-BotTox	Pro11–rabbit IgG anti-BotTox
		Pro13-goat IgG anti-BotTox

Table 2. List of interferent reagents tested

Suspicious powder	Interferent Reference Material
SP1 Spackling powder	IRM 1 Green signal smoke
SP2 Baking soda	IRM 2 Vero cell supernatant
SP3 Instant nonfat dried milk	IRM 3 Loamy Soil
SP4 Talcum powder	IRM 4 Yellow signal smoke
SP5 Flour	IRM 5 BSA fraction V
SP6 Salt	IRM 6 Water
SP7 Yeast	IRM 7 Burning vegetation
SP8 Powdered sugar	IRM 8 Burning Diesel
SP9 Dipel	IRM 9 Aspergillus niger
SP10 Chalk ($MgCO_3$)	IRM 10 Clay soil
SP11 Foot powder	IRM 11 Sage pollen
SP12 Ajax cleaner with bleach	IRM 12 Burning Fog Oil
SP13 Dairy creamer	IRM 13 Burning rubber
SP14 Kaolin	IRM 14 HC smoke / Gunshot
SP15 Bentonite	IRM 15 Sandy Soil
SP16 Aerosil	IRM 16 Violet signal smoke
	IRM 17 Red signal smoke
	IRM 18 Malathion

REFERENCES

(1) McBride, M. T.; Masquelier, D.; Hindson, B. J.; Makarewicz, A. J.; Brown, S.; Burris, K.; Metz, T.; Langlois, R. G.; Tsang, K. W.; Bryan, R.; Anderson, D. A.; Venkateswaran, K. S.; Milanovich, F. P.; Colston, B. W. *Analytical Chemistry* **2003**, *75*, 5293-5299.

(2) Hindson, B. J.; Brown, S. B.; Marshall, G. D.; McBride, M. T.; Makarewicz, A. J.; Gutierrez, D. M.; Wolcott, D. K.; Metz, T. R.; Madabhushi, R. S.; Dzenitis, J. M.; Colston, B. W. *Analytical Chemistry* **2004**, *76*, 3492-3497.

(3) Hindson, B. J.; McBride, M. T.; Makarewicz, A. J.; Henderer, B. D.; Setlur, U. S.; Smith, S. M.; Gutierrez, D. M.; Metz, T. R.; Nasarabadi, S. L.; Venkateswaran, K. S.; Farrow, S. W.; Colston, B. W.; Dzenitis, J. M. *Analytical Chemistry* **2005**, *77*, 284-289.

(4) Renzi, R. F.; Stamps, J., Horn; B. A., Ferko, S.; VanderNoot, V. A.; West, J. A. A.; Crocker, R.; Wiedenman, B.; Yee, D.; Fruetel, J. A.; *Anal. Chem.* **2005**, *77*, 435-441.

(5) Rowe-Taitt, C. A.; Golden, J. P.; Feldstein, M. J.; Cras, J. J.; Hoffman, K. E.; Ligler, F. S. *Biosensors & Bioelectronics* **2000**, *14*, 785-794.

(6) Gessler, F.; Hampe, K.; Schmidt, M.; Bohnel, H. *Diagnostic Microbiology and Infectious Disease* **2006**, *56*, 225-232.

(7) Han, S. M.; Cho, J. H.; Cho, I. H.; Paek, E. H.; Oh, H. B.; Kim, B. S.; Ryu, C.; Lee, K.; Kim, Y. K.; Paek, S. H. *Analytica Chimica Acta* **2007**, *587*, 1-8.

(8) Mangru, S.; Bentz, B. L.; Davis, T. J.; Desai, N.; Stabile, P. J.; Schmidt, J. J.; Millard, C. B.; Bavari, S.; Kodukula, K. *Journal of Biomolecular Screening* **2005**, *10*, 788-794.

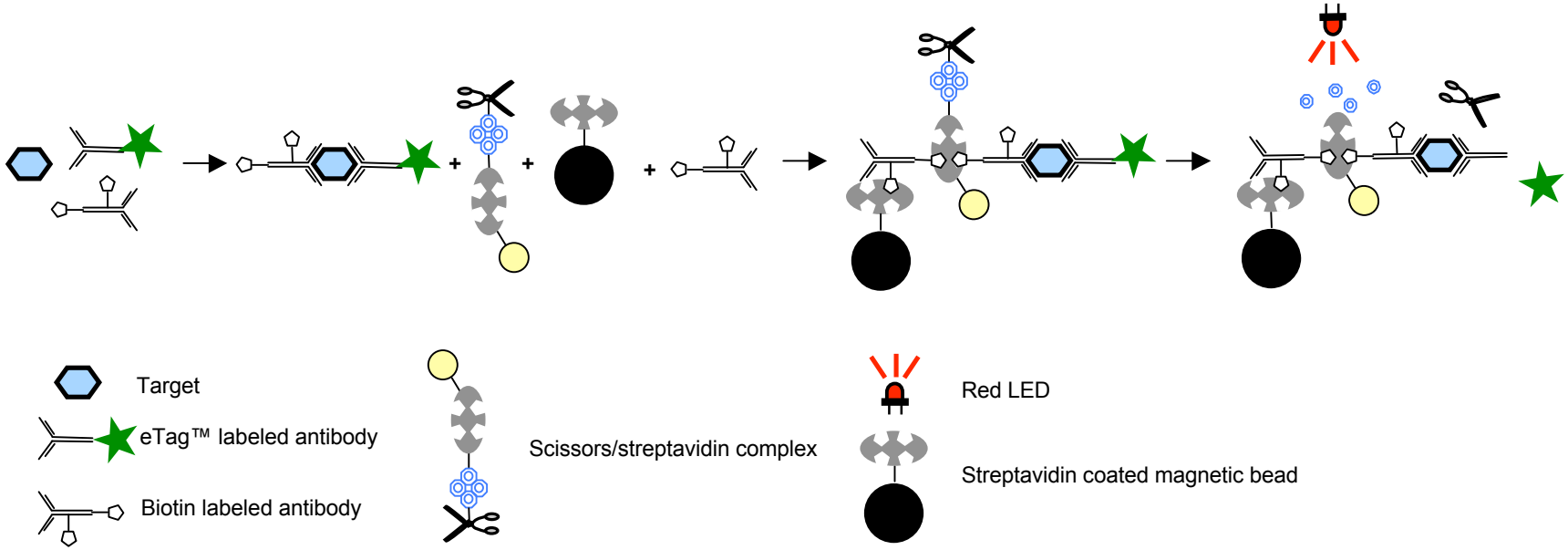
(9) Liu, W.; Montana, V.; Chapman, E. R.; Mohideen, U.; Parpura, V. *Proceedings of the National Academy of Sciences of the United States of America* **2003**, *100*, 13621-13625.

(10) Chan-Hui, P. Y.; Stephens, K.; Warnock, R. A.; Singh, S. *Clinical Immunology* **2004**, *111*, 162-174.

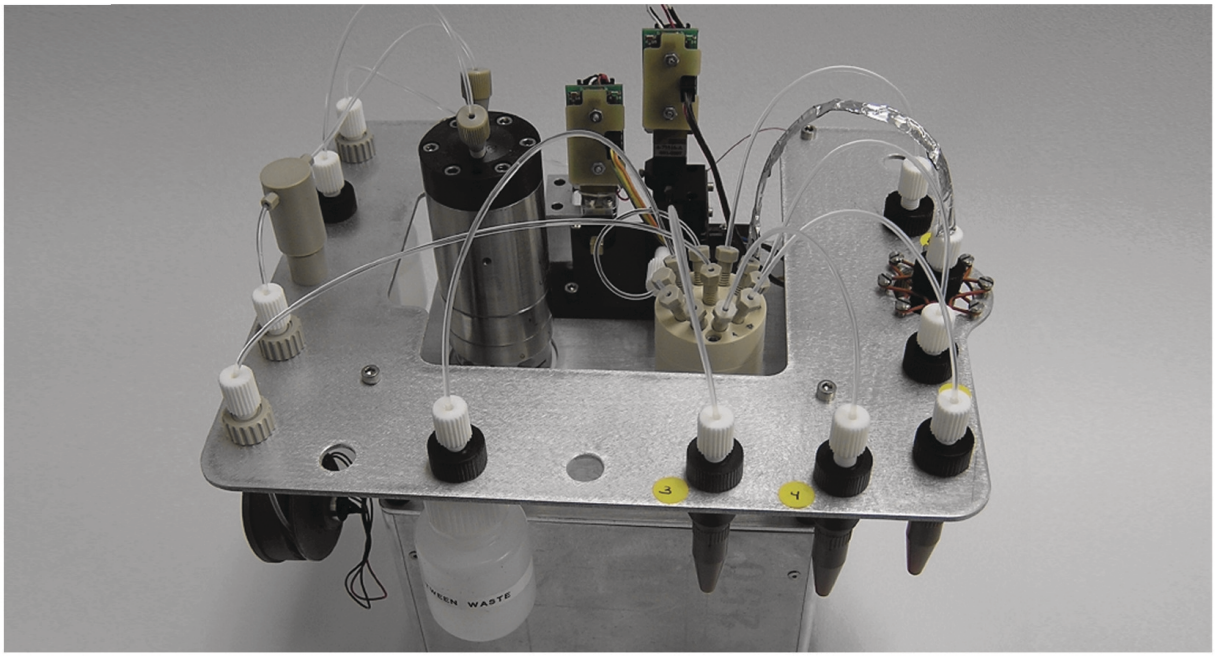
(11) Selby, C. Annals of Clinical Biochemistry **1999**, *36*, 704-721.

(12) Ligler, F. S.; Taitt, C. R.; Shriver-Lake, L. C.; Sapsford, K. E.; Shubin, Y.; Golden, J. P. *Analytical and Bioanalytical Chemistry* **2003**, *377*, 469-477.

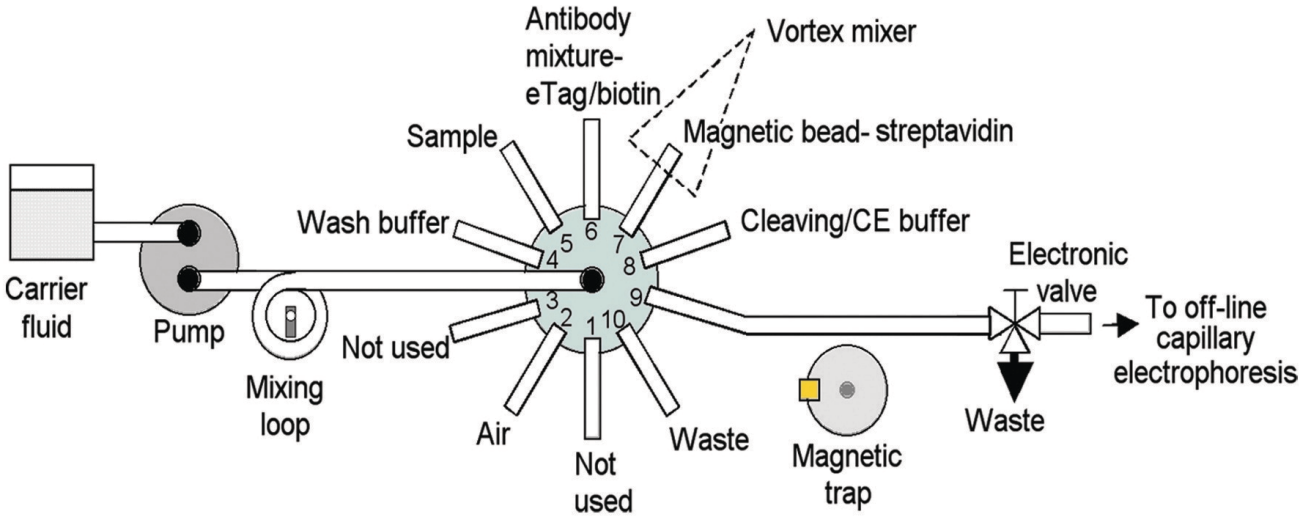
(13) King, K. D.; Vanniere, J. M.; Leblanc, J. L.; Bullock, K. E.; Anderson, G. P. *Environmental Science & Technology* **2000**, *34*, 2845-2850.


Target
eTag™ labeled antibody
Biotin labeled antibody
Scissors/streptavidin complex
Red LED
Streptavidin coated magnetic bead

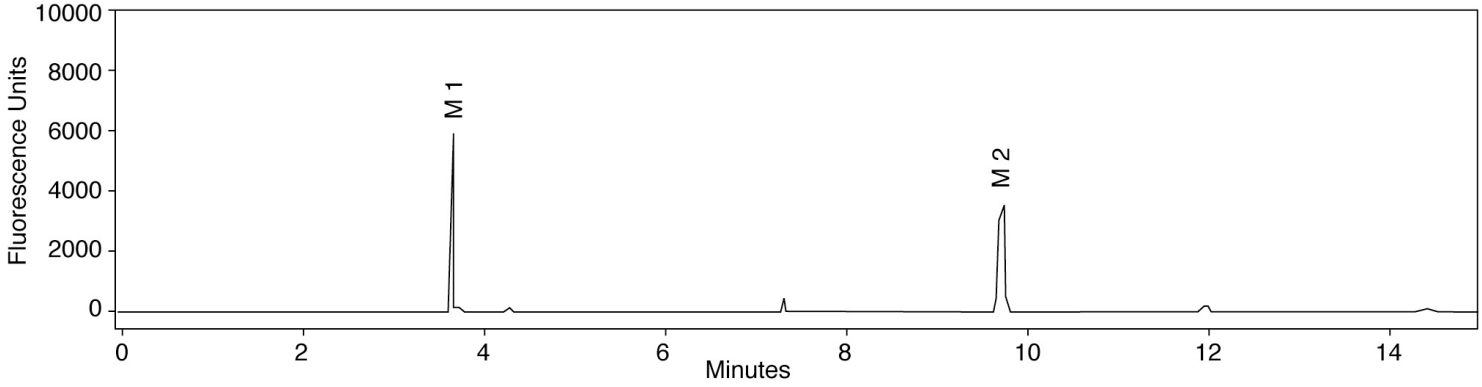
A



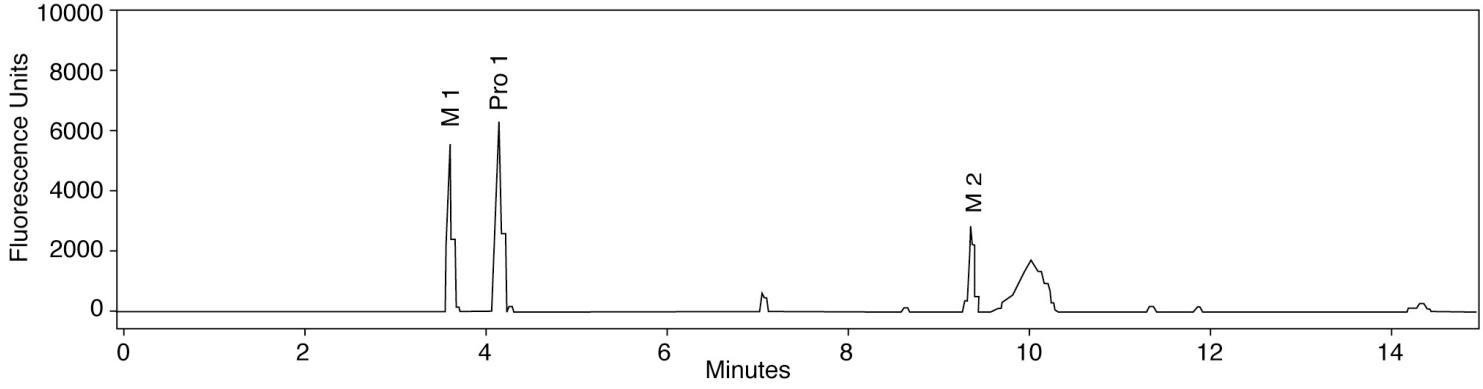
B

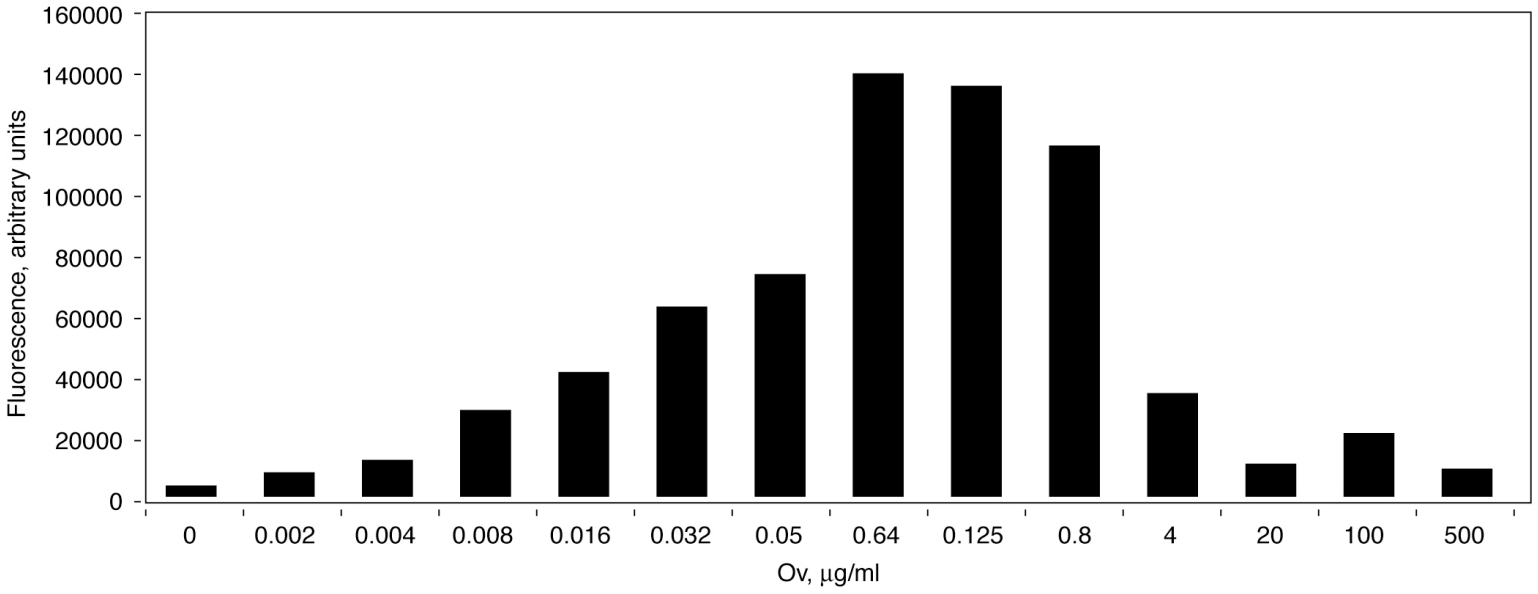

Antibody mixture- eTag/biotin
Vortex mixer
Sample
Magnetic bead- streptavidin
Wash buffer
Cleaving/CE buffer
Electronic valve
Carrier fluid
Pump
Mixing loop
Not used
To off-line capillary electrophoresis
Air
Not used
Waste
Magnetic trap
Waste
1
2
3
4
5
6
7
8
9
10

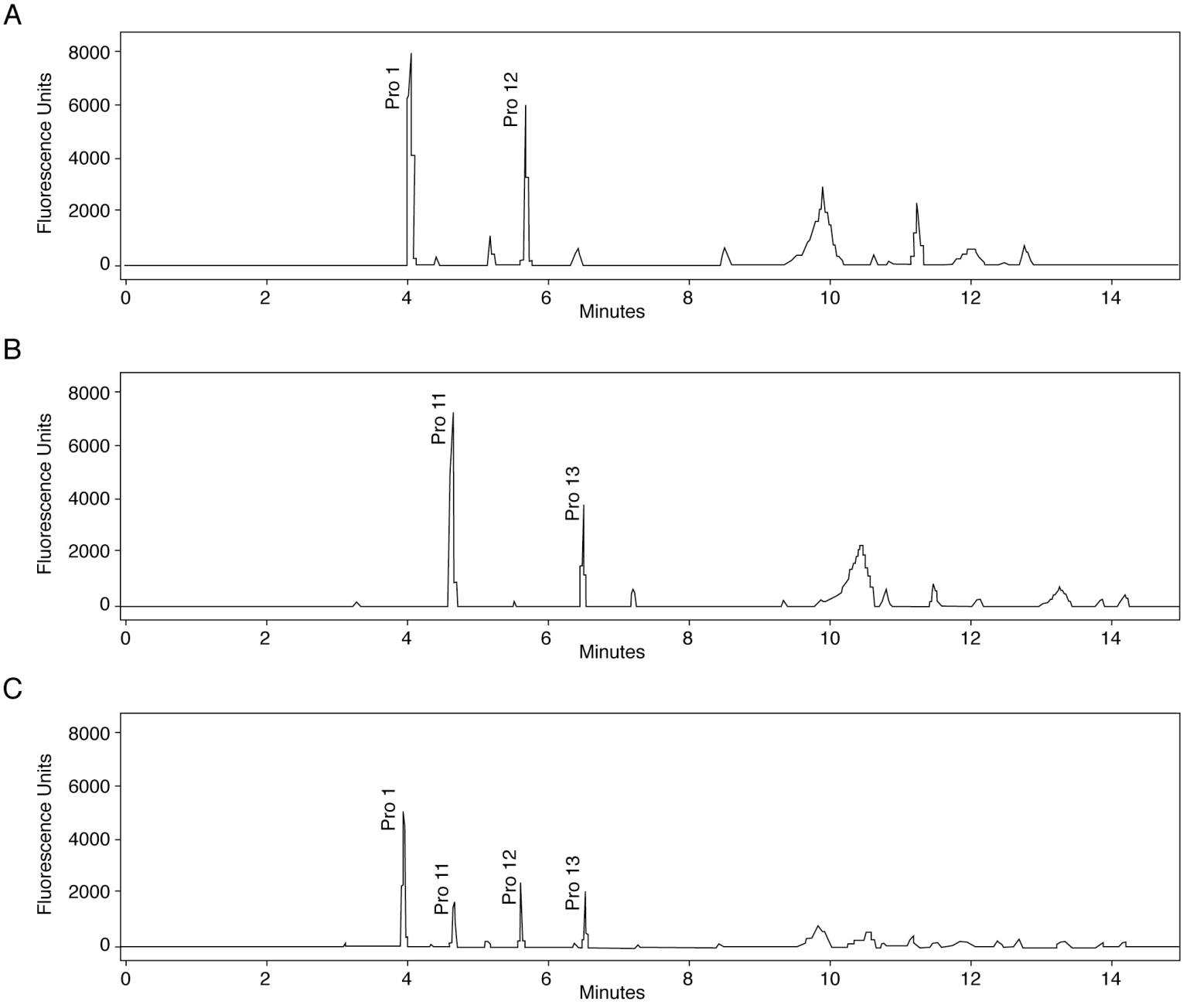
A


Fluorescence Units
10000
8000
6000
4000
2000
0
M 1
M 2
0
2
4
6
8
10
12
14
Minutes

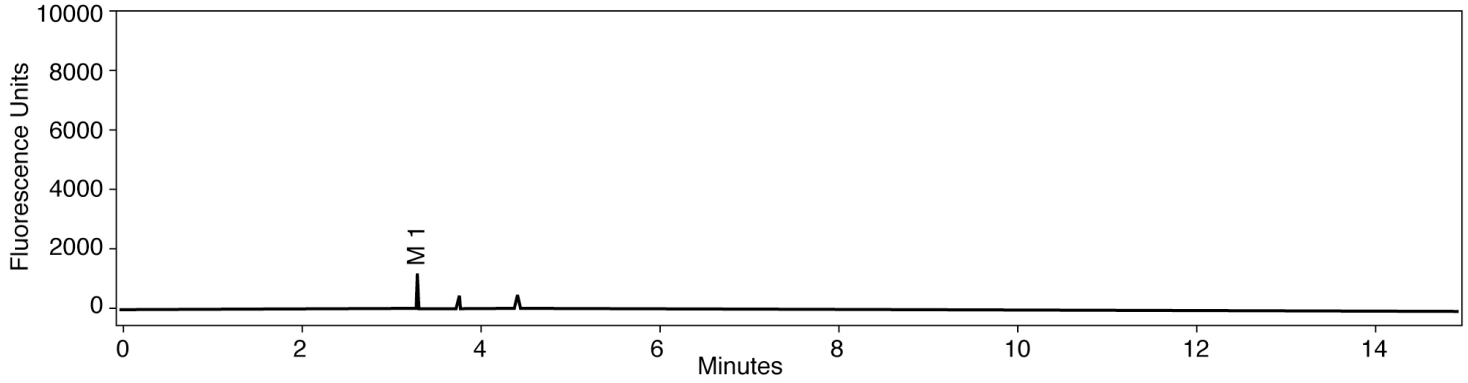
B


Fluorescence Units
10000
8000
6000
4000
2000
0
M 1
Pro 1
M 2
0
2
4
6
8
10
12
14
Minutes

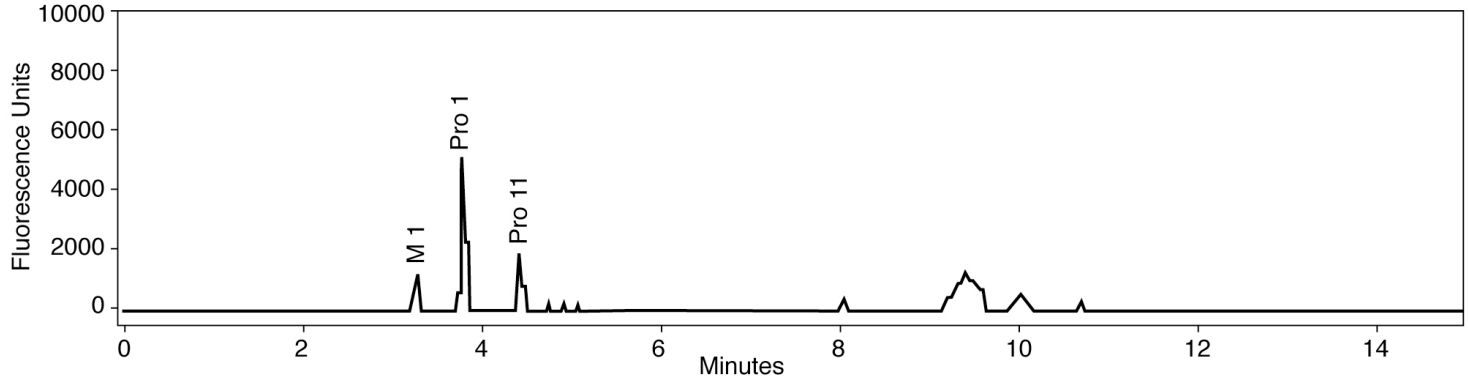

160000
140000
120000
100000
80000
60000
40000
20000
0
Fluorescence, arbitrary units
0
0.002
0.004
0.008
0.016
0.032
0.05
0.64
0.125
0.8
4
20
100
500
Ov, μg/ml


A
Fluorescence Units
8000
6000
4000
2000
0
Pro 1
Pro 12
0
2
4
6
8
10
12
14
Minutes
B
Fluorescence Units
8000
6000
4000
2000
0
Pro 11
Pro 13
0
2
4
6
8
10
12
14
Minutes
C
Fluorescence Units
8000
6000
4000
2000
0
Pro 1
Pro 11
Pro 12
Pro 13
0
2
4
6
8
10
12
14
Minutes

A


Fluorescence Units
10000
8000
6000
4000
2000
0
M 1
0
2
4
6
8
10
12
14
Minutes

B


Fluorescence Units
10000
8000
6000
4000
2000
0
M 1
Pro 1
Pro 11
0
2
4
6
8
10
12
14
Minutes